Looking for a good endothelial address

The first in vivo screening of a peptide library of the human vasculature opens new possibilities for inhibiting angiogenesis and tumor growth.

The hypothesis that tumor growth is angiogenesis-dependent (Folkman, 1971) and its subsequent confirmation by genetic methods (reviewed in Folkman, 2001a; Lyden et al., 2001) provided strong incentive for scientists to try to target peptides specifically to the vascular bed of tumors. Pasqualini and Ruoslahti achieved the first step toward this goal in 1997 when they reported a novel in vivo phage display that distinguished between active proliferating microvascular endothelial cells in a tumor and quiescent nonproliferating endothelial cells elsewhere in the vasculature (Pasqualini et al., 1997). This methodology permitted angiogenesis-related targeting of tumor blood vessels. Two years later they demonstrated that a small peptide could be specifically targeted to tumor vasculature. The peptide inhibited two metalloproteinases, resulting in inhibition angiogenesis, tumor growth, and invasion (Koivunen et al., 1999; Folkman, 1999). Now, in a landmark paper, Arap and Pasqualini and their colleagues report in vivo screening of a peptide library in a patient for the first time (Arap et al., 2002). Circulating peptides containing 47,160 motifs localized to the vasculature of different organs in a nonrandom distribution. Furthermore, certain circulating peptides bound specifically to known receptors on the vascular endothelium of the organ from which the peptide was recovered, but not to endothelium from other organs. For example, a prostate homing phage displaying a peptide mimic for interleukin-11 specifically bound to the endothelium and epithelium of normal prostate, but not to other organs such as skin. Some of the many potential clinical applications of this elegant technology were reviewed previously (Folkman, 1999). In their current report (Arap et al., 2002), the authors point out that it ultimately may become possible to determine molecular profiles of blood vessels in different organs and in specific conditions.

If such a molecular map of the human vasculature is eventually achieved and the results are taken together with the recently identified genes which encode endothelial markers overexpressed during tumor angiogenesis (St. Croix et al., 2000), a novel pharmacologic approach to angiogenesis-dependent diseases can be envisioned. Currently, antiangiogenic proteins are delivered into the circulation and achieve their high therapeutic index by selective inhibition of proliferating and migrating endothelial cells in an angiogenic focus, without having a similar effect on quiescent endothelium in the remaining vasculature. If these direct angiogenesis inhibitors, which include thrombospondin, angiostatin, endostatin and tumstatin (Maeshima et al., 2002), among others, could be targeted to the angiogenic focus in a tumor, in an atherosclerotic plaque, or in the retina or choroid of the eye, potency could be potentially enhanced.

In antiangiogenic therapy of cancer, such increased potency may be useful in the case of tumor cells deficient in p53. It has been suggested that because these tumor cells have a diminished rate of apoptosis under hypoxic conditions, that they might be less responsive to antiangiogenic therapy (Yu et al., 2002). For those angiogenesis inhibitors which have shown virtually no toxicity or side-effects in animals or humans (e.g., angiostatin, endostatin), increasing the dose or combining two or more inhibitors should obviate the problem of $p53^{-/-}$ tumor cells. Viable tumor cells form microcylinders around each capillary blood vessel that has been recruited to the tumor (Figure 1) (Folkman, 2001b). With increasing distance from the nearest blood vessel, tumor cells live under increasing hypoxia. However, beyond a given oxygen diffusion limit (which may be in the range of 110 μm for tumor cells which are $p53^{+/+}$ but greater, i.e., in the range of 150 μm, for p53 null tumor cells), anoxic conditions cause tumor cells to die. Because one endothelial cell controls the survival of approximately 50 to 100 tumor cells, a direct angiogenesis inhibitor of sufficient potency and dose to cause endothelial apoptosis would result in tumor cell death in the vessel neighborhood (Browder et al., 2000). However, for those angiogenesis inhibitors where dose cannot be increased because of the limitation of side-effects (e.g., TNP-470, a fumagillin analog [Folkman, 2001a]), targeting to the microvascular endothelium in a tumor bed may greatly increase the usefulness of the inhibitor.

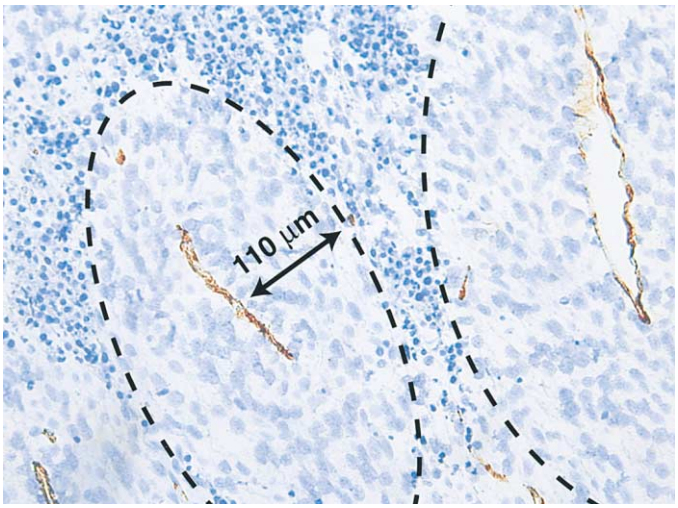

Figure 1. Histological microsection of prostate carcinoma in a rat

Viable tumor cells (light blue) form a microcylinder around each of two capillary blood vessels (highlighted orange-brown by antibody to CD31). The oxygen diffusion limit for this tumor is approximately 110 microns. Beyond that distance (interrupted line), most cells are dead due to anoxia (dark blue small cells). This figure, previously published in Folkman (2001b) is a personal communication from L. Hutchinson, L. Hlatky, and P. Hahnfeldt, and is used with permission.

Whether angiogenesis inhibitors are administered so that they reach every tissue at equivalent concentrations, or whether these agents are targeted specifically to a focus of tumor angiogenesis, their successful clinical application depends critically on an understanding of the difference between direct and indirect angiogenesis inhibitors. A direct angiogenesis inhibitor

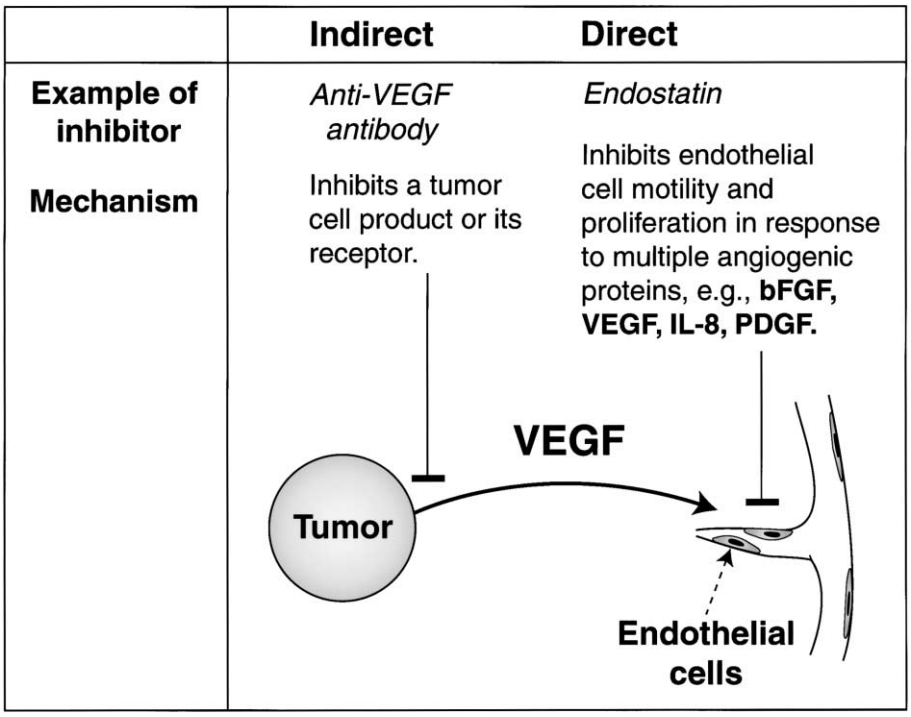

Figure 2. Diagram of indirect versus direct angiogenesis inhibitors

targets genetically stable endothelial cells recruited to a tumor (Figure 2). It inhibits or arrests their ability to proliferate, to migrate, or to grow new blood vessels. Some direct angiogenesis inhibitors, such as endostatin, cause apoptosis of growing endothelial cells (Dixelius et al., 2000). Direct angiogenesis inhibitors act independently of the cancer cell genome and are not generally subject to the problems of drug resistance, for the same reason that the genetic stability of bone marrow usually prevents it from becoming resistant to chemotherapy. In contrast, an indirect angiogenesis inhibitor generally inhibits a tumor cell product or its receptor (Figure 3). For example, an antibody against a tumor-generated pro-angiogenic protein, VEGF (vascular endothelial growth factor), may successfully inhibit angiogenesis induced by a tumor releasing only or mainly VEGF (Figure 3). However, if mutant tumor cells develop which produce other pro-angiogenic proteins, such as bFGF (basic fibroblast growth factor) or TGFβ-1 (transforming growth factor β-1) (Figure 3), the tumor may resume its angiogenic activity and appear to become resistant to the indirect inhibitor. In women first diagnosed with breast cancer, approximately 60% of tumors produce only VEGF. In subsequent recurrences or in metastases, additional angiogenic proteins are found (Relf et al., 1997). An indirect angiogenesis inhibitor which blocks only VEGF might be effective early after diagnosis, but might be less effective in treating later recurrences because these tumors are producing multiple pro-angiogenic proteins. On the other hand, some indirect angiogenesis inhibitors may bring about complete and durable regression of a tumor that has failed all conventional chemotherapy or radiotherapy, if the angiogenic activity in that tumor is mediated by a pro-angiogenic protein which remains the same throughout treatment. For example, certain high grade giant cell tumors and angioblastomas in humans in which angiogenesis is mediated only or mainly by bFGF have been eradicated by treatment with interferon-α, which blocks production of bFGF (Singh et al., 1995) by tumor cells (Figure 3) (Marler et al., 2002).

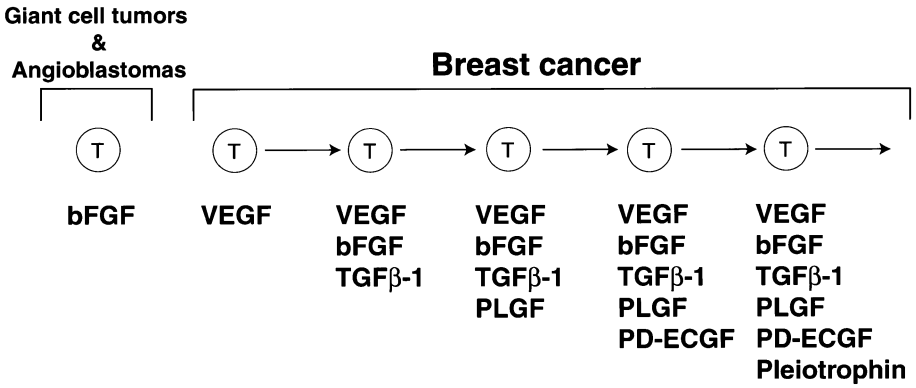

Figure 3. Diagram to illustrate increase in different types of pro-angiogenic proteins released by human breast cancers (T = tumor) with passage of time (arrows) (Relf et al., 1997)

In contrast, certain tumors, such as high grade giant cell tumors and angioblastomas (left), continue to produce only or mainly bFGF (Marler et al., 2002).

The in vivo screening of peptide libraries in patients may have numerous other applications that cannot be perceived at this early stage of its development. For example, such screening may provide clues to predict metastatic patterns, or it may even be used to detect the targets of circulating endothelial cells and stem cells, or to direct these cells to a prespecified target. This novel approach to detection of addresses in the vascular system may also be used in early detection of neurologic diseases such as Alzheimer's disease. For cancer therapy, however, it is not clear if clinicians will always be as dependent on knowing the precise location of a cancer as they are today, because of increasing development of molecular diagnosis. Nevertheless, molecular diagnosis depends to a large extent on detection of molecules shed from a tumor or its vascular bed into a body fluid. A molecular map of the human vasculature may come to play a pivotal role in the development of molecular diagnosis. If molecular diagnosis of cancer from blood, urine, stool, and other body fluids continues to develop, and if the toxicity of cancer therapy can be significantly reduced, there may come a time when a patient

with a positive molecular marker for cancer will be treated until the marker becomes negative, perhaps without visualization of the tumor or its metastases. This scenario is beginning to happen in recurrent ovarian cancer.

Cancer therapy today is anchored to a taxonomy of a tumor's site of origin and its location (Andrew von Eschenbach, personal communication). One can speculate that someday, this paradigm may shift to a therapy heavily based on molecular markers where site of origin may not be essential (i.e., for antiangiogenic therapy), and where the need to know the location of a tumor may not be required. Such a change in medical practice would recapitulate the history of the treatment of infection. Before antibiotics, it was critical to know the location of an infection so that pus could be drained; after antibiotics, the treatment of many infections could be guided by markers in the blood. If cancer can someday be treated as a chronic manageable disease, analogous to heart disease or diabetes, the report of Arap and Pasqualini and their colleagues (Arap et al., 2002), in addition to its heuristic value, will have made a major contribution toward this clinical goal.

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